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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 January 2001 (15.01.01)	
International application No. PCT/GB00/01740	**Applicant's or agent's file reference** P021661WO
International filing date (day/month/year) 05 May 2000 (05.05.00)	**Priority date** (day/month/year) 10 May 1999 (10.05.99)
Applicant BRUNE, Martin, Hermann, Klemens et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   07 December 2000 (07.12.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P021661WO		
Int rnational application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 01740	05/05/2000	10/05/1999
Applicant		
MEDICAL RESEARCH COUNCIL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rul 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from th date of mailing of this int rnational search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 7

   [X] as suggested by th applicant.

   [ ] Non of th figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better charact rizes the inv ntion.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]B 00/01740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/48 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 52467 A (UNIV DUNDEE ;MEHTA ANIL (GB); MUIMO RICHMOND (GB)) 8 September 2000 (2000-09-08) claims 1-3 page 2, line 14-17 page 16, line 21 -page 18, line 13 page 24, line 22 - line 25 ---	1-3,10
X	US 5 741 635 A (GUHA ABHIJIT ET AL) 21 April 1998 (1998-04-21) claims 1,2,5 column 3, line 41 - line 44 column 8, line 45 -column 9, line 6 column 9, line 49 -column 10, line 1 column 10, line 35 - line 51 column 11, line 46 - line 63 --- -/--	1-3,8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2000	13/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]GB 00/01740

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 923 796 A (DENEKE ULFERT ET AL) 8 May 1990 (1990-05-08) claims column 5, line 61 -column 6, line 14 table 1 examples 7-10	1-3,8-10
X	US 4 806 415 A (FOSSATI PIERO) 21 February 1989 (1989-02-21) column 3, line 60 - line 64 column 4, line 56 - line 58	1-3,8-10
X	FR 2 660 933 A (PASTEUR INSTITUT) 18 October 1991 (1991-10-18) claims 1-4,7 page 2, line 29 - line 35 page 3, line 10 - line 23	1-3,8-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/01740

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0052467	A	08-09-2000	NONE		
US 5741635	A	21-04-1998	NONE		
US 4923796	A	08-05-1990	DE	2834704 A	21-02-1980
			AT	362532 B	25-05-1981
			AT	426079 A	15-10-1980
			AU	514885 B	05-03-1981
			AU	4927579 A	14-02-1980
			CA	1129316 A	10-08-1982
			DD	145328 A	03-12-1980
			DE	2960533 D	29-10-1981
			DK	315679 A	09-02-1980
			EP	0009076 A	02-04-1980
			JP	1171163 C	17-10-1983
			JP	55026896 A	26-02-1980
			JP	58004915 B	28-01-1983
			YU	191479 A	31-08-1984
US 4806415	A	21-02-1989	IT	1172385 B	18-06-1987
			AT	45983 T	15-09-1989
			AU	555501 B	25-09-1986
			AU	3492884 A	27-06-1985
			CA	1227113 A	22-09-1987
			DE	3479594 D	05-10-1989
			EP	0147713 A	10-07-1985
			JP	1615821 C	30-08-1991
			JP	2040320 B	11-09-1990
			JP	60156400 A	16-08-1985
FR 2660933	A	18-10-1991	EP	0498849 A	19-08-1992
			WO	9106671 A	16-05-1991

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/01740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/48 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 52467 A (UNIV DUNDEE ;MEHTA ANIL (GB); MUIMO RICHMOND (GB)) 8 September 2000 (2000-09-08) claims 1-3 page 2, line 14-17 page 16, line 21 -page 18, line 13 page 24, line 22 - line 25 ---	1-3,10
X	US 5 741 635 A (GUHA ABHIJIT ET AL) 21 April 1998 (1998-04-21) claims 1,2,5 column 3, line 41 - line 44 column 8, line 45 -column 9, line 6 column 9, line 49 -column 10, line 1 column 10, line 35 - line 51 column 11, line 46 - line 63 --- -/--	1-3,8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2000	13/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate. of the relevant passages	Relevant to claim No.
X	US 4 923 796 A (DENEKE ULFERT ET AL) 8 May 1990 (1990-05-08) claims column 5, line 61 -column 6, line 14 table 1 examples 7-10	1-3,8-10
X	US 4 806 415 A (FOSSATI PIERO) 21 February 1989 (1989-02-21) column 3, line 60 - line 64 column 4, line 56 - line 58	1-3,8-10
X	FR 2 660 933 A (PASTEUR INSTITUT) 18 October 1991 (1991-10-18) claims 1-4,7 page 2, line 29 - line 35 page 3, line 10 - line 23	1-3,8-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/01740

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0052467	A	08-09-2000	NONE		
US 5741635	A	21-04-1998	NONE		
US 4923796	A	08-05-1990	DE	2834704 A	21-02-1980
			AT	362532 B	25-05-1981
			AT	426079 A	15-10-1980
			AU	514885 B	05-03-1981
			AU	4927579 A	14-02-1980
			CA	1129316 A	10-08-1982
			DD	145328 A	03-12-1980
			DE	2960533 D	29-10-1981
			DK	315679 A	09-02-1980
			EP	0009076 A	02-04-1980
			JP	1171163 C	17-10-1983
			JP	55026896 A	26-02-1980
			JP	58004915 B	28-01-1983
			YU	191479 A	31-08-1984
US 4806415	A	21-02-1989	IT	1172385 B	18-06-1987
			AT	45983 T	15-09-1989
			AU	555501 B	25-09-1986
			AU	3492884 A	27-06-1985
			CA	1227113 A	22-09-1987
			DE	3479594 D	05-10-1989
			EP	0147713 A	10-07-1985
			JP	1615821 C	30-08-1991
			JP	2040320 B	11-09-1990
			JP	60156400 A	16-08-1985
FR 2660933	A	18-10-1991	EP	0498849 A	19-08-1992
			WO	9106671 A	16-05-1991

CARPMAELS & RANSFORD

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JANDAN M ALISS† ROBERT M C CARPMAEL*

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DEREK G R GRUNDY S DAVID VOTIER OBE

* Patent Attorney † Trade Mark Attorney

European Patent Office
Erhardtstrasse 27
D-80298 Munich
GERMANY

Attn: N Favre

YOUR REF

OUR REF P021661WO/HGH/CJM

19th June 2001

Dear Sirs,

Re: International patent application PCT/GB00/01740
Medical Research Council

Following the telephone interview which took place on 6th June 2001, amended claims for this patent application are enclosed. I am extremely grateful to the examiner for the helpful way in which examination of this application has been handled.

Only claims 1 and 2 have been amended. These have been amended in the same way as each other and the amendment finds clear basis at page 2, lines 14-15. These are the amendments which Mr Marshall suggested in the telephone interview.

In making this amendment, any subject-matter which may no longer be claimed has not been abandoned in any way. It may be reinstated in the present application or may be used as the basis of one or more divisional applications.

I look forward to receiving the IPER in due course.

Yours truly,

HALLYBONE, Huw George

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HALLYBONE, Huw George
CARPMAELS & RANSFORD
43 Bloomsbury Square
London WC1A 2RA
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Applicant's or agent's file reference P021661WO:HGH	Date of mailing *(day/month/year)* 01.08.2001
	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01740	05/05/2000	10/05/1999

Applicant
MEDICAL RESEARCH COUNCIL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Digiusto, M Tel.+49 89 2399-8162

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P021661WO:HGH		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01740	05/05/2000	10/05/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/48		
Applicant MEDICAL RESEARCH COUNCIL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/12/2000	01.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01740

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-14	as originally filed				

Claims, No.:

1-19	as received on	20/06/2001	with letter of	19/06/2001	

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (US-A-5 741 635) refers to methods of detecting GTP and GDP. In one embodiment, D1 discloses (e.g. column 3, lines 40-44) a method where GDP is converted to detectable GTP by phosphorylation, i.e. by dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).

   Document D2 (US-A-4 923 796) discloses (e.g. column 5, line 61 - column 6, line 6) a method where GTP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) ADP in a sample.

   Similarly, document D3 (US-A-4 806 415) discloses (e.g. column 4, line 56 -57) an method where ADP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) GTP in a sample.

1.1 Independent claims 1 and 2 define processes for detecting the presence of a nucleoside diphosphate or triphosphate in a sample, said processes comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a NDPK. Said processes differ for the teachings of D1-D3 in that said processes measure a change of the enzyme **itself**, and not an increase or decrease of the product.

1.2 The problem to be solved by the present invention might thus be seen as the provision of an alternative method to that of D1-D3.

1.3 Document D4 (FR-A-2 660 933) discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, the teachings of D4 addresses the detection of tumour cells, said detection using the quantification of the NDPK.

Moreover, the substrates used in D4 cannot be hydrolysed (page 3, lines 14-17) and therefore cannot be used in the methods of claims 1 and 2 or in the methods taught by D1-D3.
Hence, none of the prior art documents at hand discloses or fairly suggests the methods of claims 1 or 2. The subject-matter of independent claims 1 and 2 thus meets the requirements of Articles 33(2) and 33(3) PCT.

1.4 Dependent claims 3-11 further define specific embodiments of the novel and inventive methods of claims 1 and 2. Moreover, dependent claims 4-7 and 11 define methods which differ from those disclosed in the prior art documents at hand in that the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated. None of the prior art documents at hand discloses or fairly suggests such methods.
Dependent claims 3-11 hence also meet the requirements of Articles 33(2) and 33(3) PCT.

2. Document D4 discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, none of the prior art documents at hand discloses or fairly suggests a NDPK carrying a label, for instance a fluorescent label as defined in claim 13, wherein the label itself gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated
The subject-mater of claims 12 and 13 is thus novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

2.1 Furthermore, none of the prior art document at hand discloses or fairly suggests the covalent attachment of a fluorescent label to the NDPK via a cysteine residue. Moreover, the prior art documents at hand do not disclose or fairly suggest a NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate.
Therefore, the subject-matter of claims 14-17 is also considered to meet the

requirements of Articles 33(2) and 33(3) PCT.

2.3 According to the above argumentation, none of the prior art documents at hand discloses or fairly suggests the binding of the enzymes of claims 12-17 to a substrate or using said enzymes as an *in vivo* or *in vitro* diagnostic reagent. Independent claims 18 and 19 thus also meet the requirements of Articles 33(2) and 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-00 52467	08.09.2000	02.03.2000	02.03.1999*

Should the present application enter the national or regional phase, the above cited document could be relevant for the question of novelty (see e.g. page 16, line 21 - page 18, line 13).

*Validity of the claimed priority has not yet been checked.

Re Item VIII
Certain observations on the international application

1. Although claims 12 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is

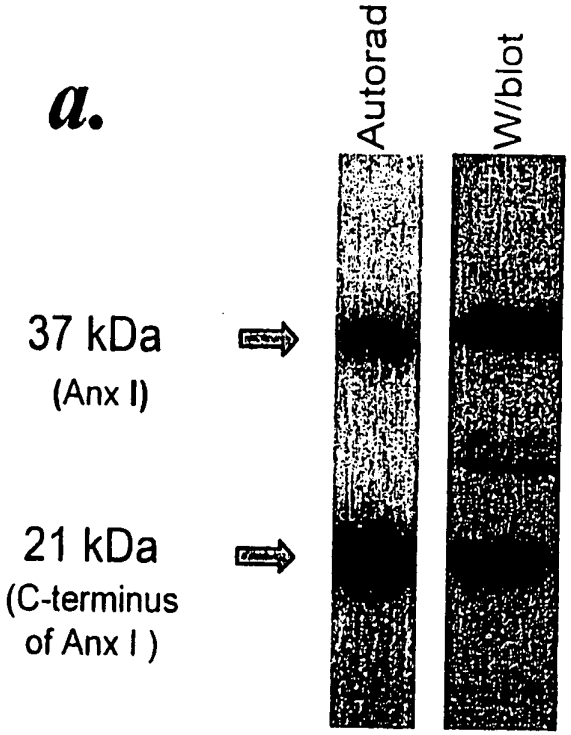
sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 12 and 16 do not meet the requirements of Article 6 PCT.

2. The vague and imprecise statement in the description on page 13, lines 28-29, implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

3. Finally, for the sake of clarity (Article 6 PCT), it might be, in view of dependent claim 4, advantageous to include the wording "which may be modified to carry a label" in the wording of present independent claims 1 and 2, i.e. "... of a nucleoside diphosphate kinase (NDPK), which may be modified to carry a label, by detecting a change ...".

Fig. 14

a.



b.

SEELAVNDDLADSAR (188-204) (SEQ ID No.5)
VLDLELKGDIEK (251-262) (SEQ ID No.6)

c.

```
                230       240       250       260       270       280       290
                 |         |     *   |         |         |         |       |   *
Bovine      ..... RSYPHLRRVFQKYSKYSKHDMNKVLDLELKGDIEKCLTVIVKCATSQPMFFAEKLHQAMKGIGTRHK...
Human       ..... RSYPQLRRVFQKYTKYSKHDMNKVLDLELKGDIEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHK...
Mouse       ..... RSFPHLRRVFQNYGKYSQHDMNKALDLELKGDIEKCLTTIVKCATSTPAFFAEKLYEAMKGAGTRHK...
Rabbit      ..... RSYLHLRRVFQKYSKYSQHDMNKVLDLELKGDIEKCLTAIVQCATCKPAYFAEKLYQAMKGAGTRHK...
```

INTERNATIONAL SEARCH REPORT

Int[illegible]al Application No
PCT/GB 00/00736

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/50 C12Q1/48 G01N33/577 G01N33/573 C07K7/08
C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RIEMEN, C.E. ET AL.: "Defective nucleoside triphosphate synthesis in plasma membranes derived from the tracheal epithelium of CFTR null (-/-) mice" THE JOURNAL OF PHYSIOLOGY, vol. 509, no. 3, 15 June 1998 (1998-06-15), page 81p XP000914291 cited in the application the whole document --- -/--	1-42

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 June 2000	04/07/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Gundlach, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TREHARNE, K.J. ET AL.: "A novel chloride-dependent GTP-utilizing protein kinase in plasma membranes from human respiratory epithelium" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 267, no. 5, November 1994 (1994-11), pages L592-L601, XP000914274 cited in the application the whole document	1-42
A	ANCIAUX, K. ET AL.: "Inhibition of nucleoside diphosphate kinase (NDPK/nm23) by cAMP analogues" FEBS LETTERS, vol. 400, 1997, pages 75-79, XP000914537 cited in the application the whole document	1-42
A	MARSHALL, L.J. ET AL.: "Na+ and K+ regulate the phosphorylation state of nucleoside diphosphate kinase in human airway epithelium" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 276, January 1999 (1999-01), pages c109-c119, XP000920864 cited in the application the whole document	1-42
A	TSAO, F.H.C. ET AL.: "Degradation of Annexin I in Bronchoalveolar Lavage Fluid from Patients with Cystic Fibrosis" AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY, vol. 18, no. 1, January 1998 (1998-01), pages 120-128, XP000914301 cited in the application the whole document	1-50
A	US 5 051 364 A (ISACKE CLARE M ET AL) 24 September 1991 (1991-09-24) the whole document	43-50

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. [redacted]l Application No
PCT/GB 00/00736

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9616084 A	30-05-1996	US 5877179 A	02-03-1999
		AU 698762 B	05-11-1998
		AU 4146096 A	17-06-1996
		CN 1176648 A	18-03-1998
		EP 0794963 A	17-09-1997
		JP 10509953 T	29-09-1998
		NZ 296765 A	25-02-1999
US 5051364 A	24-09-1991	NONE	

'ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P021661WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 01740	05/05/2000	10/05/1999
Applicant		
MEDICAL RESEARCH COUNCIL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 7

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
GB 00/01740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/48 C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 52467 A (UNIV DUNDEE ;MEHTA ANIL (GB); MUIMO RICHMOND (GB)) 8 September 2000 (2000-09-08) claims 1-3 page 2, line 14-17 page 16, line 21 -page 18, line 13 page 24, line 22 - line 25 ---	1-3,10
X	US 5 741 635 A (GUHA ABHIJIT ET AL) 21 April 1998 (1998-04-21) claims 1,2,5 column 3, line 41 - line 44 column 8, line 45 -column 9, line 6 column 9, line 49 -column 10, line 1 column 10, line 35 - line 51 column 11, line 46 - line 63 --- -/--	1-3,8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2000	13/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/GB 00/01740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 923 796 A (DENEKE ULFERT ET AL) 8 May 1990 (1990-05-08) claims column 5, line 61 -column 6, line 14 table 1 examples 7-10	1-3,8-10
X	US 4 806 415 A (FOSSATI PIERO) 21 February 1989 (1989-02-21) column 3, line 60 - line 64 column 4, line 56 - line 58	1-3,8-10
X	FR 2 660 933 A (PASTEUR INSTITUT) 18 October 1991 (1991-10-18) claims 1-4,7 page 2, line 29 - line 35 page 3, line 10 - line 23	1-3,8-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
GB 00/01740

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0052467	A	08-09-2000	NONE		
US 5741635	A	21-04-1998	NONE		
US 4923796	A	08-05-1990	DE	2834704 A	21-02-1980
			AT	362532 B	25-05-1981
			AT	426079 A	15-10-1980
			AU	514885 B	05-03-1981
			AU	4927579 A	14-02-1980
			CA	1129316 A	10-08-1982
			DD	145328 A	03-12-1980
			DE	2960533 D	29-10-1981
			DK	315679 A	09-02-1980
			EP	0009076 A	02-04-1980
			JP	1171163 C	17-10-1983
			JP	55026896 A	26-02-1980
			JP	58004915 B	28-01-1983
			YU	191479 A	31-08-1984
US 4806415	A	21-02-1989	IT	1172385 B	18-06-1987
			AT	45983 T	15-09-1989
			AU	555501 B	25-09-1986
			AU	3492884 A	27-06-1985
			CA	1227113 A	22-09-1987
			DE	3479594 D	05-10-1989
			EP	0147713 A	10-07-1985
			JP	1615821 C	30-08-1991
			JP	2040320 B	11-09-1990
			JP	60156400 A	16-08-1985
FR 2660933	A	18-10-1991	EP	0498849 A	19-08-1992
			WO	9106671 A	16-05-1991

ATP

Main assays are coupled enzyme using NADH change. Lots of minor variations, based on:

Lamprecht, W., I. Trautschold. 1965. Adenosine-5'-triphosphate. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: H. U. Bergmeyer, editor. Methods of Enzymatic Analysis. 2nd ed. New York: Academic Press. p 2101-2110.

And using luciferase

Strehler B. L. 1965. Adenosine-5'-triphosphate and creatine phosphate determination with luciferase. In: H. U. Bergmeyer, editor. Methods of Enzymatic Analysis. 2nd ed. New York: Academic Press. p 2112-2126.

Karamohamed S., G. Guidotti. 2001. Bioluminometric method for real-time detection of ATPase activity. *BioTechniques* 31:420-425.

ADP

Main assay using coupled enzymes and NADH change. Lots of minor variations, based on:

Jaworek D., W. Gruber, H. U. Bergmeyer. 1965. Adenosine-5'-diphosphate and adenosine-5'-monophosphate. In: H. U. Bergmeyer, editor. Methods of Enzymatic Analysis. 2nd ed. New York: Academic Press. p 2127-2131.

One example;

Takashi R., S. Putnam. 1979. A fluorimetric method for continually assaying ATPase: application to small specimens of glycerol-extracted muscle fibers. *Anal. Biochem.* 92:375-382.

Also NMR

Williams S. P., A. M. Fulton, K. M. Brindle. 1993. Estimation of the intracellular free ADP concentration by 19F NMR studies of fluorine-labeled yeast phosphoglycerate kinase in vivo. *Biochemistry* 32:4895-4902.

Presumably also electrochemical based assays, but I have no leads

Fluorescence labeling of proteins to form a sensor

None relate to ADP or ATP nor can I find anything (above what is already in patent documents) on using a phosphoenzyme as a basis for a sensor.

Brune M., J. L. Hunter, J. E. T. Corrie, M. R. Webb. 1994. Direct, real-time measurement of rapid inorganic phosphate release using a novel fluorescent probe and its application to actomyosin subfragment 1 ATPase. *Biochemistry* 33:8262-8271.

Zhou L. Q., A. E. G. Cass. 1991. Periplasmic binding protein based biosensors 1. Preliminary study of the maltose binding protein as sensing element for maltose biosensor. *Biosensors and Bioelectronics* 6:445-450.

Marvin J. S., H. W. Hellinga. 1998. Engineering biosensors by introducing fluorescent allosteric signal transducers: construction of a novel glucose sensor. *J. Am. Chem. Soc.* 120:7-11.

Richieri G. V., A. Anel, A. M. Kleinfeld. 1993. Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* 32:7574-7580.

Schauer-Vukasinovic V., L. Cullen, S. Daunert. 1997. Rational design of a calcium sensing system based on induced conformation changes in calmodulin. *J. Am. Chem. Soc.* 119:11102-11103.

Honda A., S. R. Adams, C. L. Sawyer, V. Lev-Ram, R. Y. Tsien, W. R. Dostmann. 2001. Spatiotemporal dynamics of guanosine 3',5'-cyclic monophosphate revealed by a genetically encoded, fluorescent indicator. *Proc. Natl. Acad. Sci. U.S.A.* 98:2437-2442.

Miyawaki A., J. Llopis, R. Heim, J. M. McCaffery, J. A. Adams, M. Ikura, R. Y. Tsien. 1997. Fluorescent indicators for Ca2+ based on green fluorescent proteins and calmodulin. *Nature* 388:882-887.

09/937296

PATENT COOPERATION TREATY

PCT

REC'D 06 AUG 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P021661WO:HGH		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01740	05/05/2000	10/05/1999

International Patent Classification (IPC) or national classification and IPC
C12Q1/48

RECEIVED
JAN 17 2002
TECH CENTER 1600/2900

Applicant
MEDICAL RESEARCH COUNCIL et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/12/2000	01.08.2001

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Favre, N
Telephone No. +49 89 2399 7363

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01740

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-14	as originally filed				

Claims, No.:

1-19	as received on	20/06/2001	with letter of	19/06/2001	

Drawings, sheets:

1/7-7/7	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (US-A-5 741 635) refers to methods of detecting GTP and GDP. In one embodiment, D1 discloses (e.g. column 3, lines 40-44) a method where GDP is converted to detectable GTP by phosphorylation, i.e. by dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).

   Document D2 (US-A-4 923 796) discloses (e.g. column 5, line 61 - column 6, line 6) a method where GTP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) ADP in a sample.

   Similarly, document D3 (US-A-4 806 415) discloses (e.g. column 4, line 56 -57) an method where ADP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) GTP in a sample.

1.1 Independent claims 1 and 2 define processes for detecting the presence of a nucleoside diphosphate or triphosphate in a sample, said processes comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a NDPK. Said processes differ for the teachings of D1-D3 in that said processes measure a change of the enzyme **itself**, and not an increase or decrease of the product.

1.2 The problem to be solved by the present invention might thus be seen as the provision of an alternative method to that of D1-D3.

1.3 Document D4 (FR-A-2 660 933) discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, the teachings of D4 addresses the detection of tumour cells, said detection using the quantification of the NDPK.

Moreover, the substrates used in D4 cannot be hydrolysed (page 3, lines 14-17) and therefore cannot be used in the methods of claims 1 and 2 or in the methods taught by D1-D3.
Hence, none of the prior art documents at hand discloses or fairly suggests the methods of claims 1 or 2. The subject-matter of independent claims 1 and 2 thus meets the requirements of Articles 33(2) and 33(3) PCT.

1.4 Dependent claims 3-11 further define specific embodiments of the novel and inventive methods of claims 1 and 2. Moreover, dependent claims 4-7 and 11 define methods which differ from those disclosed in the prior art documents at hand in that the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated. None of the prior art documents at hand discloses or fairly suggests such methods.
Dependent claims 3-11 hence also meet the requirements of Articles 33(2) and 33(3) PCT.

2. Document D4 discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, none of the prior art documents at hand discloses or fairly suggests a NDPK carrying a label, for instance a fluorescent label as defined in claim 13, wherein the label itself gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated
The subject-mater of claims 12 and 13 is thus novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

2.1 Furthermore, none of the prior art document at hand discloses or fairly suggests the covalent attachment of a fluorescent label to the NDPK via a cysteine residue. Moreover, the prior art documents at hand do not disclose or fairly suggest a NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate.
Therefore, the subject-matter of claims 14-17 is also considered to meet the

requirements of Articles 33(2) and 33(3) PCT.

2.3 According to the above argumentation, none of the prior art documents at hand discloses or fairly suggests the binding of the enzymes of claims 12-17 to a substrate or using said enzymes as an *in vivo* or *in vitro* diagnostic reagent. Independent claims 18 and 19 thus also meet the requirements of Articles 33(2) and 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-00 52467	08.09.2000	02.03.2000	02.03.1999*

Should the present application enter the national or regional phase, the above cited document could be relevant for the question of novelty (see e.g. page 16, line 21 - page 18, line 13).

*Validity of the claimed priority has not yet been checked.

Re Item VIII
Certain observations on the international application

1. Although claims 12 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is

sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 12 and 16 do not meet the requirements of Article 6 PCT.

2. The vague and imprecise statement in the description on page 13, lines 28-29, implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

3. Finally, for the sake of clarity (Article 6 PCT), it might be, in view of dependent claim 4, advantageous to include the wording "which may be modified to carry a label" in the wording of present independent claims 1 and 2, i.e. "... of a nucleoside diphosphate kinase (NDPK), which may be modified to carry a label, by detecting a change ...".

CLAIMS (Article 34 PCT)

1. A process for detecting the presence of a nucleoside diphosphate in a sample, comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK) by detecting a change in a characteristic of the NDPK which differs between its phosphorylated and unphosphorylated forms.

2. A process for detecting the presence of a nucleoside triphosphate in a sample, comprising the step of detecting the phosphorylation of a nucleoside diphosphate kinase (NDPK) to the phosphoenzyme form by detecting a change in a characteristic of the NDPK which differs between its phosphorylated and unphosphorylated forms.

3. The process of claim 1 or claim 2, wherein the phosphorylation or dephosphorylation is detected by using an intrinsic property of NDPK.

4. The process of claim 1 or claim 2, wherein the NDPK is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

5. The process of claim 4, wherein the NDPK carries a fluorescent label.

6. The process of claim 5, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

7. The process of claim 5 or claim 6, wherein the fluorescent label is IDCC (*N*-[2-(iodoacetamido)ethyl]-7-diethylaminocoumarin-3-carboxamide).

8. The process of claim 1, wherein the nucleoside diphosphate is ADP or GDP.

9. The process of claim 2, wherein the nucleoside triphosphate is ATP or GTP.

10. The process of any preceding claim, being a quantitative process.

11. The process of any preceding claim, wherein the NDPK is the NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

12. NDPK which is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

13. The NDPK of claim 12, wherein the label on the modified NDPK is a fluorescent label.

14. The NDPK of claim 13, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

15. The NDPK of claim 13 or claim 14, wherein the fluorescent label is IDCC.

16. NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

17. NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate

18. A substrate having the NDPK of any one of claims 12 to 17 immobilised thereto.

19. The NDPK of any one of claims 12 to 17 for use as an *in vivo* or *in vitro* diagnostic reagent.

(WZ: 26-4-01

PATENT COOPERATION TREATY

VCTM

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PTO/PCT Rec'd 25 SEP 2001

To:

HALLYBONE, Huw George
CARPMAELS & RANSFORD
43 Bloomsbury Square
London WC1A 2RA
GRANDE BRETAGNE

RECEIVED
29 JAN 200
CARPMAELS & RANSFORD
ACTIONED ..SW..

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	26.01.2001
Applicant's or agent's file reference: P021661WO:HGH	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01740	05/05/2000	10/05/1999

International Patent Classification (IPC) or both national classification and IPC

C12Q1/48

Applicant

MEDICAL RESEARCH COUNCIL et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 10/09/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Favre, N

Formalities officer (incl. extension of time limits)
Danti, B
Telephone No. +49 89 2399 8161

I. Basis of the opini n

1 - This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

Description, pages:

1-14 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrialapplicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3, 8-10 and 12
Inventive step (IS)	Claims	13, 18 and 19
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R It m V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (US-A-5 741 635) refers to methods of detecting GTP and GDP. In one embodiment, D1 discloses (e.g. column 3, lines 40-44) a method where GDP is converted to detectable GTP by phosphorylation, i.e. by dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).

   Document D2 (US-A-4 923 796) discloses (e.g. column 5, line 61 - column 6, line 6) a method where GTP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) ADP in a sample.

   Similarly, document D3 (US-A-4 806 415) discloses (e.g. column 4, line 56 -57) an method where ADP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) GTP in a sample.

   Finally, in the method disclosed in D4 (FR-A-2 660 933, e.g. page 6, line 3 - page 7, line 28) TDP is converted to TTP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) TTP in a sample.

1.1 Independent claims 1 and 2 define processes for detecting the presence of a nucleoside diphosphate or triphosphate in a sample, said processes comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a NDPK.
   In view of the above arguments, the subject-matter of independent claims 1 and 2 is not novel in the sense of Article 33(2) PCT over the disclosures of D1-D4.

1.2 Given that the phosphorylation respectively the dephosphorylation of nucleoside, i.e dephosphorylation of the phosphoenzyme and phosphorylation of the enzyme, respectively, is an intrinsic feature of NDPK, the subject-matter defined in dependent claim 3 is not novel. Moreover, the methods defined in dependent claims 8-10 do not contain any features which renders the therein defined

methods different from the disclosures of D1-D4.
Therefore, dependent claims 3 and 8-10 do not meet the requirements of Article 33(2) PCT.

1.3 However, dependent claims 4-7 and 11 define methods which differ from those disclosed in the prior art documents at hand in that the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.
None of the prior art documents at hand fairly suggests such methods. Claims 4-7 and 11 thus fulfill the requirements of Articles 33(2) and 33(3) PCT.

2. Document D4 discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive).
The subject-mater of claims 12 is thus not novel in the sense of Article 33(2) PCT.

2.1 The use of a fluorescent label instead of a radioactive label is merely a straight-forward possibility the skilled person would select without the exercise of inventive skill. Thus, dependent claim 13 does not contain any features which, in combination with the features of claim 12, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT).

2.2 However, none of the prior art document at hand discloses or fairly suggests the covalent attachment of a fluorescent label to the NDPK via a cysteine residue. Moreover, the prior art documents at hand do not disclose or fairly suggest a NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate.
Therefore, the subject-matter of claims 14-17 is considered to meet the requirements of Articles 33(2) and 33(3) PCT.

2.3 Binding know enzymes to a substrate and using known enzymes as an *in vivo* or *in vitro* diagnostic reagent does not require an inventive activity from the person

skilled in the art.
Independent claims 18 and 19 thus do not meet the requirements of Article 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-00 52467	08.09.2000	02.03.2000	02.03.~~2000~~* 1999

Should the present application enter the national or regional phase, the above cited document could be relevant for the question of novelty (see e.g. page 16, line 21 - page 18, line 13).

*Validity of the claimed priority has not been checked.

Re Item VIII
Certain observations on the international application

1. Although claims 12 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims

makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 12 and 16 do not meet the requirements of Article 6 PCT.

2. The vague and imprecise statement in the description on page 13, lines 28-29, implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

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Erhardtstrasse 27
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GERMANY
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YOUR REF

OUR REF P021661WO/HGH/CJM

19th March 2001

Dear Sirs,

Re: International patent application PCT/GB00/01740
Medical Research Council

The applicant's comments on the written opinion issued on 26th January 2001 in connection with the above-mentioned patent application are presented below.

The examiner has argued that:

- process claims 1-3 and 8-10 lack novelty over each of D1, D2, D3 and D4
- product claim 12 lacks novelty over D4
- product claims 13, 18 and 19 are obvious over D4

These objections are incorrect.

Claims 1 and 2 read as follows:

> *1. A process for detecting the presence of a nucleoside diphosphate in a sample, comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).*
>
> *2. A process for detecting the presence of a nucleoside triphosphate in a sample, comprising the step of detecting the phosphorylation of a nucleoside diphosphate kinase (NDPK) to the phosphoenzyme form.*

As set out on page 2, lines 11-15, of the application as filed, these processes will involve the step of detecting a change in a property of the enzyme which differs between its phosphorylated (NDPK~P) and unphosphorylated (NDPK) forms. An essential step in this process, therefore, is that the phosphorylation state of the enzyme is measured –

where NDPs are being detected, reduction of phosphoenzyme levels (or consequent increase of NDPK levels) is measured; where NTPs are being detected, the production of phosphoenzyme (or removal of unphosphorylated enzyme) is measured.

In both cases, however, the process requires the observation of either (a) NDPK~P dephosphorylation, or (b) NDPK phosphorylation. It is an inherent feature of the claimed invention, therefore, that the levels of NDPK and/or of NDPK~P are measured before and after (or during) contact with the sample of interest. A key feature of the present invention is thus the change in the NDPK:NDPK~P ratio.

D1 discloses methods for assaying GDP or GTP bound to a G protein. The passage in column 3 which has been cited by the examiner simply confirms that GDP can be converted to GTP using NDPK – the enzyme is used simply to convert GDP to GTP, and the GTP is then assayed using standard methods. There is no mention of measuring the NDPK:NDPK~P ratio. Claims 1 and 2 are therefore novel over D1.

D2 discloses a method which uses the kinase to convert ADP to ATP. The ATP is then used to produce NADH or NADPH, which is used as an indirect measure of the initial amount of ADP. Like D1, however, it does not disclose the idea of measuring levels of NDPK in comparison with NDPK~P, and claims 1 and 2 are therefore novel over D2.

D3 is similar, disclosing a method assaying ATP. Again, however, the enzyme is used simply for its known activity – there is no mention of measuring changes in phosphoenzyme levels.

Looking at this another way, none of documents D1, D2 or D3 mentions the existence of the phosphoenzyme form of NDPK. This alone means that the steps of "detecting the dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK)" (claim 1) and of "detecting the phosphorylation of a nucleoside diphosphate kinase (NDPK) to the phosphoenzyme form" (claim 2) must be novel.

D4 is different from D1 to D3, because it relates to methods for assaying levels of NDPK enzyme, rather than levels of NDP/NTP. This means, however, that D4 does not disclose, therefore, a "process for detecting the presence of a nucleoside diphosphate [or triphosphate] in a sample", but discloses a "process for detecting the presence of NDPK in a sample". Whilst D4 does disclose that NDPK~P levels could be measured, this is only disclosed in the context of using an 'suicide' γ-thiophosphate derivative (*e.g.* claim 3; page 3, lines 10-17) – there is no disclosure of forming NDPK~P using native NTP. D4 does not suggest, therefore, that levels of NDPK~P phosphoenzyme could be used to indicate the presence of NDP or NTP in a sample. Claims 1 and 2 are therefore novel over D4.

In considering the above arguments, the examiner should also note the statement on page 3, lines 3-5, of the present application:

> *The invention is based on the finding that the phosphoenzyme intermediate is stable over a time-scale that allows its detection and measurement.*

None of D1 to D4 discloses this stability of NDPK~P, so none of D1 to D4 discloses or suggests the process of claim 1 or claim 2. Nor is the idea of using isolated phosphoenzyme as a reagent (page 3, lines 15-20) disclosed.

The examiner has also cited WO00/52467 (D5), which claims an earlier priority date that the present application. Like D1 to D4, however, this document also fails to disclose that measurement of NDPK phosphorylation levels would allow NDP/NTP levels to be detected or measured in a sample. The purpose of the assay on pages 16-18 of D5 is to determine whether NDPK is functional, not to detect nucleoside phosphates. Thus D5 is also irrelevant to novelty.

To summarise, therefore, the prior art uses NDPK simply as a means of catalysing the transfer of γ-phosphate from a NTP to a NDP, and not as a means of detection. The level of NDPK phosphorylation is irrelevant in these assays, but is an essential aspect of the present invention as defined in the claims.

Moving now to the product claims, claim 12 reads:

> *12. NDPK which is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.*

The NDPK is D4 is not modified within the meaning of claim 12.

The examiner's view is that the ^{32}P-phosphoenzyme disclosed in D4 falls within the scope of claim 12, but this is incorrect. The examiner's argument requires the ^{32}P atom to be the "label" of claim 12. However, ^{32}P cannot give a "different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated" because ^{32}P gives the <u>same</u> detectable signal whether it is attached to the enzyme or not.

Put another way, claim 12 requires the label to be "carried" by the enzyme in both the NDPK and NDPK~P forms, but ^{32}P is <u>removed</u> in dephosphorylation. ^{32}P cannot give "a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated" because it is not present in the unphosphorylated enzyme. The claim requires the label to be carried whatever the phosphorylation state of the enzyme, with a differential signal being detected. ^{32}P does not satisfy this requirement, because this "label" is only present in the phosphoenzyme – the "label" is not carried by the unphosphorylated enzyme.

Put differently again, the examiner's argument implies that claim 12 lacks novelty over two separate enzymes in D4, namely NDPK <u>and</u> NDPK~^{32}P. Whilst these two proteins certainly give "different detectable signals", this is because they are different proteins, neither of which in isolation falls within claim 12 – the claim is novel over the NDPK of D4 and is also novel over the NDPK~^{32}P of D4.

The failure of the examiner's argument also becomes apparent when claim 13 is considered. The examiner believes that "the use of a fluorescent label instead of a radioactive label is merely a straightforward possibility". Looking at D4, however, if a

fluorescent label is used instead of the ^{32}P "label", there will be no formation of phosphoenzyme. The examiner's reasoning would require some way of labelling phosphate with a fluorescent label, rather than the NDPK enzyme itself, but this is not possible.

It is therefore clear that the requirements of claim 12 are not satisfied by the ^{32}P-phosphoenzyme disclosed in D4, because the ^{32}P atom does not function as a "label" within the meaning of the claim. Claim 12 is therefore novel over D4.

In view of the above, it is apparent that the claims as originally filed satisfy the requirements of Articles 33(2) and 33(3) PCT. An IPER acknowledging this would be welcomed.

If the Examiner does not agree with the arguments set forth above and is minded to issue an unfavourable IPER, however, a further opportunity to submit arguments or amendments is requested [Rule 66.4(b)] and the option of a telephone discussion with the Examiner is requested [Article 34(2)(a)].

Yours truly,

HALLYBONE, Huw George

CJM

PATENT COOPERATION TREATY

FAX: 020-7405 4166

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HALLYBONE, Huw George
CARPMAELS & RANSFORD
43 Bloomsbury Square
London WC1A 2RA
GRANDE BRETAGNE

CONFIRMATION

RECEIVED
15 JUN 2001
CARPMAELS & RANSFORD
ACTIONED .....

PCT

NOTIFICATION CONCERNING INFORMAL COMMUNICATIONS WITH THE APPLICANT

(PCT Rule 66.6)

Date of mailing *(day/month/year)*	07.06.2001
Applicant's or agent's file reference P021661WO:HGH	**REPLY DUE** within 1 month(s) from the above date of mailing
International application no. PCT/GB00/01740	International filing date *(day/month/year)* 05/05/2000
Applicant MEDICAL RESEARCH COUNCIL et al.	

An informal communication took place on 05/06/2001, between the International Preliminary Examining Authority and the applicant / the agent.

Invitation pursuant to Rules 66.2 c), 66.3 and 66.4 of the PCT

Further examination of the international application has revealed that the application fails to meet the requirements of the PCT and the Regulations as explained in the attached note (Form PCT/IPEA/428).

The Applicant is hereby **invited**, within the time limit indicated above, **to submit a written reply** accompanied by amendments.

If no reply is submitted, the international preliminary examination report will reflect the opinion expressed by this Authority.

Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Digiusto, M Telephone No.+49 89 2399-8162

Form PCT/IPEA/429 (july 1993)

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traité de coopération en matière de brevets

PCT

Application No.: PCT/GB00/01740

Note on an informal communication by telephone with the Applicant

Transmittal of a copy of this note with a time limit of **1 month(s)**

Participants

Agent: Hallybone, HG / Marshall, CJ

Examiner(s): Favre, N

Summary of the communication

The objections presented in the written opinion have been discussed in the light of the arguments presented in the letter of reply dated 19.05.2001.

It has been agreed that the introduction of the statement of page 2, lines 14-15, into the wording of present claims 1 and 2 would confer novelty and inventive step to the subject-matter of said claims.

As agreed, the next action to be taken is the filing of an amended set of claims by the applicant within the time limit set herein-above.

05/06/2001
.....
Date *(day / month / year)*

Favre, N
.....
Authorized officer of IPEA

CLAIMS

1. A process for detecting the presence of a nucleoside diphosphate in a sample, comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).

2. A process for detecting the presence of a nucleoside triphosphate in a sample, comprising the step of detecting the phosphorylation of a nucleoside diphosphate kinase (NDPK) to the phosphoenzyme form.

3. The process of claim 1 or claim 2, wherein the phosphorylation or dephosphorylation is detected by using an intrinsic property of NDPK.

4. The process of claim 1 or claim 2, wherein the NDPK is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

5. The process of claim 4, wherein the NDPK carries a fluorescent label.

6. The process of claim 5, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

7. The process of claim 5 or claim 6, wherein the fluorescent label is IDCC (*N*-[2-(iodoacetamido)ethyl]-7-diethylaminocoumarin-3-carboxamide).

8. The process of claim 1, wherein the nucleoside diphosphate is ADP or GDP.

9. The process of claim 2, wherein the nucleoside triphosphate is ATP or GTP.

10. The process of any preceding claim, being a quantitative process.

11. The process of any preceding claim, wherein the NDPK is the NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

12. NDPK which is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

13. The NDPK of claim 12, wherein the label on the modified NDPK is a fluorescent label.

14. The NDPK of claim 13, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

15. The NDPK of claim 13 or claim 14, wherein the fluorescent label is IDCC.

16. NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

17. NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate

18. A substrate having the NDPK of any one of claims 12 to 17 immobilised thereto.

19. The NDPK of any one of claims 12 to 17 for use as an *in vivo* or *in vitro* diagnostic reagent.

- The location of a phosphate (*i.e.* either bound to NDPK, or as the γ-phosphate of a NTP) can be ascertained by following the ^{31}P NMR spectrum.
- Protons whose environment changes upon dephosphorylation can be detected by, for instance, NMR.
- Dephosphorylation may cause a change in the fluorescence of a tryptophan residue in the protein [*e.g.* ref 18].
- Dephosphorylation can be detected by following the loss of ^{32}P from radio-labelled phosphoenzyme. The radio-isotope can be conveniently incorporated into NDPK by using [γ-^{32}P]ATP.
- Circular dichroism, or any other suitable spectrometric technique, can detect conformational changes which occur on dephosphorylation.
- Dephosphorylation may result in a change in surface plasmon resonance properties.

Rather than using properties inherent in the wild-type enzyme, it may be desired to modify the enzyme in some way. This may also be important where dephosphorylation of the NDPK of choice does not exhibit an intrinsic measurable change which can be readily followed.

One particularly preferred modification is the addition of a fluorescent label to the enzyme, typically via a cysteine residue. If the wild-type protein lacks a suitable cysteine residue (*e.g.* the NDPK of *Myxococcus xanthus* (SEQ ID NO:1)), this can easily be introduced by mutagenesis [*e.g.* 19]. A suitable position for mutation can easily be determined by the skilled person, whilst ensuring that the mutation does not disrupt the enzymatic activity [*e.g.* 20]. At any given amino acid residue, particular labels may give better results than others. Suitable combinations of label and residue can be determined by routine experimentation.

Preferred fluorescent labels are based around coumarin. Particularly preferred is *N*-[2-(iodoacetamido)ethyl]-7-diethylaminocoumarin-3-carboxamide [21; Fig. 1], referred to simply as 'IDCC' hereafter. This is preferably attached to a cysteine residue, and preferably exhibits a high fluorescence when NDPK is phosphorylated, and a low fluorescence when NDPK is dephosphorylated. When suitably attached to NDPK, this label offers the advantage that the phosphoenzyme can detect small quantities of ADP in the presence of much higher concentrations of ATP. This is extremely important for experiments in situations where ATP levels are high *e.g.* in single muscle fibres. It is also able to respond very quickly to changes in ADP levels, and gives a large signal change over a range of several hundred micromolar.

crystal structure has been determined [16,27]. The wild-type sequence does not contain any cysteine, so the gene was manipulated to introduce cysteine residues by site-directed mutagenesis in *E.coli* strains TG1 and DH5α using either a phosphothioate-based method [28, produced in kit form by Amersham] or the PCR-based QuikChange kit [Stratagene].

Using the Amersham kit, the 0.8 kb *HindIII-EcoRI* fragment of pJM5C2A [29] containing the *ndk* gene from *M.xanthus* was ligated with M13mp19, and the resulting recombinant clones were used to provide single-stranded DNA templates for mutagenesis. For cloning, the mutated *ndk* genes were cloned back into pJM5C2A. As an alternative, the 0.7 kb *BstXI-EcoRI* fragment of the M13ndk constructs was ligated into a modified form of the Invitrogen™ pRSetA expression vector, whose coding sequence for a histidine tag fused to the N-terminus of NDPK had been removed. This yielded the 3.5 kb pRSndkX series of plasmids, where the final "X" is a number in a series of *ndk* mutations.

pRSndk was also used as a template for the QuikChange method.

Various mutant proteins containing cysteine residues were prepared, including D112C (*i.e.* Asp-112 was mutated to Cys) and D62C. Positions for mutation were typically chosen on the basis of their proximity to the nucleotide-binding cleft seen in the crystal structure [16].

The mutant D112C gene was produced in plasmid pRSndk4, which was also used for expression. For best results, freshly-transformed cells were used for starter cultures. 200µl calcium-competent BL21 cells [Novagen] were incubated with 2ng pRSndk4 plasmid DNA for 30 minutes on ice. Half of this mixture was then spread onto an LB agar plate containing 0.1mg/ml ampicillin and incubated overnight at 37°C. Plates typically contained 50-100 colonies. 100ml LB medium containing 0.1mg/ml ampicillin was inoculated with 2-3 colonies from the plate and grown for 9 hours at 37°C until cells had just entered stationary phase. For the main culture, 8x500ml LB+ampicillin was inoculated with 10ml started culture and incubated at 37°C for 6 hours, after which time the cells had typically reached OD_{595} of 0.38. At this point, 0.5mg/ml IPTG was added to each flask, and the cells grown for a further 16 hours. Cells were harvested by centrifugation in a Beckman L2 centrifuge at 3800rpm and 20°C for 20 minutes. The pellet was resuspended in 100ml Buffer A (20mM Tris-HCl, pH 8.2, 1mM EDTA) and stored at –80°C.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P021661WO:HGH		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/01740	05/05/2000	10/05/1999

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International Patent Classification (IPC) or national classification and IPC
C12Q1/48

Applicant
MEDICAL RESEARCH COUNCIL et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/12/2000	01.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich	Authorized officer Fouss, N

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (US-A-5 741 635) refers to methods of detecting GTP and GDP. In one embodiment, D1 discloses (e.g. column 3, lines 40-44) a method where GDP is converted to detectable GTP by phosphorylation, i.e. by dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK).

   Document D2 (US-A-4 923 796) discloses (e.g. column 5, line 61 - column 6, line 6) a method where GTP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) ADP in a sample.

   Similarly, document D3 (US-A-4 806 415) discloses (e.g. column 4, line 56 -57) an method where ADP is converted to ATP (by dephosphorylation of the phosphoenzyme form of a NDPK) in order to determine the level of (detect) GTP in a sample.

1.1 Independent claims 1 and 2 define processes for detecting the presence of a nucleoside diphosphate or triphosphate in a sample, said processes comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a NDPK. Said processes differ for the teachings of D1-D3 in that said processes measure a change of the enzyme **itself**, and not an increase or decrease of the product.

1.2 The problem to be solved by the present invention might thus be seen as the provision of an alternative method to that of D1-D3.

1.3 Document D4 (FR-A-2 660 933) discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, the teachings of D4 addresses the detection of tumour cells, said detection using the quantification of the NDPK.

Moreover, the substrates used in D4 cannot be hydrolysed (page 3, lines 14-17) and therefore cannot be used in the methods of claims 1 and 2 or in the methods taught by D1-D3.
Hence, none of the prior art documents at hand discloses or fairly suggests the methods of claims 1 or 2. The subject-matter of independent claims 1 and 2 thus meets the requirements of Articles 33(2) and 33(3) PCT.

1.4 Dependent claims 3-11 further define specific embodiments of the novel and inventive methods of claims 1 and 2. Moreover, dependent claims 4-7 and 11 define methods which differ from those disclosed in the prior art documents at hand in that the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated. None of the prior art documents at hand discloses or fairly suggests such methods.
Dependent claims 3-11 hence also meet the requirements of Articles 33(2) and 33(3) PCT.

2. Document D4 discloses (cf. page 3, lines 10-16) a method for the quantisation of NDPK by radioactively labelling the enzyme using ATP or GTP analogues, i.e. the enzyme is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated (radioactive) from when it is unphosphorylated (not radioactive). However, none of the prior art documents at hand discloses or fairly suggests a NDPK carrying a label, for instance a fluorescent label as defined in claim 13, wherein the label itself gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated
The subject-mater of claims 12 and 13 is thus novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

2.1 Furthermore, none of the prior art document at hand discloses or fairly suggests the covalent attachment of a fluorescent label to the NDPK via a cysteine residue. Moreover, the prior art documents at hand do not disclose or fairly suggest a NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate.
Therefore, the subject-matter of claims 14-17 is also considered to meet the

CLAIMS (Article 34 PCT)

1. A process for detecting the presence of a nucleoside diphosphate in a sample, comprising the step of detecting the dephosphorylation of the phosphoenzyme form of a nucleoside diphosphate kinase (NDPK) by detecting a change in a characteristic of the NDPK which differs between its phosphorylated and unphosphorylated forms.

2. A process for detecting the presence of a nucleoside triphosphate in a sample, comprising the step of detecting the phosphorylation of a nucleoside diphosphate kinase (NDPK) to the phosphoenzyme form by detecting a change in a characteristic of the NDPK which differs between its phosphorylated and unphosphorylated forms.

3. The process of claim 1 or claim 2, wherein the phosphorylation or dephosphorylation is detected by using an intrinsic property of NDPK.

4. The process of claim 1 or claim 2, wherein the NDPK is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

5. The process of claim 4, wherein the NDPK carries a fluorescent label.

6. The process of claim 5, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

7. The process of claim 5 or claim 6, wherein the fluorescent label is IDCC (*N*-[2-(iodoacetamido)ethyl]-7-diethylaminocoumarin-3-carboxamide).

8. The process of claim 1, wherein the nucleoside diphosphate is ADP or GDP.

9. The process of claim 2, wherein the nucleoside triphosphate is ATP or GTP.

10. The process of any preceding claim, being a quantitative process.

11. The process of any preceding claim, wherein the NDPK is the NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

12. NDPK which is modified to carry a label which gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated.

14. The NDPK of claim 13, wherein the fluorescent label is attached to the NDPK via a cysteine residue.

15. The NDPK of claim 13 or claim 14, wherein the fluorescent label is IDCC.

16. NDPK of *Myxococcus xanthus* carrying a Asp112→Cys mutation, and carrying an IDCC label at this mutated residue.

17. NDPK modified by the attachment of at least one detectable label that is sensitive to the binding of a nucleoside diphosphate

18. A substrate having the NDPK of any one of claims 12 to 17 immobilised thereto.

19. The NDPK of any one of claims 12 to 17 for use as an *in vivo* or *in vitro* diagnostic reagent.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01740

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-14	as originally filed			

Claims, No.:

1-19	as received on	20/06/2001	with letter of	19/06/2001

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-19
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-19
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-19
	No:	Claims

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

requirements of Articles 33(2) and 33(3) PCT.

2.3 According to the above argumentation, none of the prior art documents at hand discloses or fairly suggests the binding of the enzymes of claims 12-17 to a substrate or using said enzymes as an *in vivo* or *in vitro* diagnostic reagent. Independent claims 18 and 19 thus also meet the requirements of Articles 33(2) and 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO-A-00 52467	08.09.2000	02.03.2000	02.03.1999*

Should the present application enter the national or regional phase, the above cited document could be relevant for the question of novelty (see e.g. page 16, line 21 - page 18, line 13).

*Validity of the claimed priority has not yet been checked.

Re Item VIII
Certain observations on the international application

1. Although claims 12 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is

sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 12 and 16 do not meet the requirements of Article 6 PCT.

2. The vague and imprecise statement in the description on page 13, lines 28-29, implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

3. Finally, for the sake of clarity (Article 6 PCT), it might be, in view of dependent claim 4, advantageous to include the wording "which may be modified to carry a label" in the wording of present independent claims 1 and 2, i.e. "... of a nucleoside diphosphate kinase (NDPK), which may be modified to carry a label, by detecting a change ...".